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David Saliwanchik

David R. Saliwanchik, Patent Attorney

REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 CFR 1.322
Docket No. CIB-T100XC1
Patent No. 6,870,075

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Peter R. Beetham, Patricia L. Avissar, Keith A. Walker, Richard A. Metz
Issued	:	March 22, 2005
Patent No.	:	6,870,075
For	:	Methods of Making Non-Transgenic Herbicide Resistant Plants

Mail Stop Certificate of Corrections Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION
UNDER 37 CFR 1.322 (OFFICE MISTAKE)

Sir:

A Certificate of Correction (in duplicate) for the above-identified patent has been prepared and is attached hereto.

In the left-hand column below is the column and line number where errors occurred in the patent. In the right-hand column is the page and line number in the application where the correct information appears.

Patent Reads:	**Application Reads:**
Column 1, line 60:	Page 2, line 6:
"higher K_1"	--higher K_i--

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Column 2, line 7:	Page 2, line 15:
"apparent K_I"	--apparent K_i--
Column 3, line 42:	Page 4, line 13:
"to an non-transgenic"	--to a nontransgenic--
Column 5, line 23:	Page 6, line 19:
"development"	--developmental--
Column 8, line 65:	Page 11, line 4:
"such as"	--such an--
Column 51, line 23 (Claim 7):	Page 3 of Examiner's Amendment (12/24/03), claim 21, line 3:
"Met_{178}"	-- Met_{176}--
Column 52, line 9 (Claim 12):	Page 4 of Examiner's Amendment (12/24/03), claim 27, line 3:
"Leu_{189}"	--Leu_{169}--

True and correct copies of pages 2, 4, 6, 11 of the application as originally filed, as well as copies of pages 3 and 4 of the Examiner's Amendment dated December 24, 2003, which support Applicants' assertion of errors on the part of the Patent Office, accompany this Certificate of Correction.

Approval of the Certificate of Correction is respectfully requested.

Respectfully submitted,

David Saliwanchik

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Attachments: Certificate of Correction;
Copies of pages 2, 4, 6, 11 of application as originally filed;
Copies of pages 3 and 4 of Examiner's Amendment (12/24/03)

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 6,870,075 Page 1 of 1

DATED : March 22, 2005

INVENTORS : Peter R. Beetham, Patricia L. Avissar, Keith A. Walker, Richard A. Metz

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 1,
Line 60, "higher K_1" should read -- higher K_i--.

Column 2,
Line 7, "apparent K_1" should read -- apparent K_i--.

Column 3,
Line 42, "to an non-transgenic" should read -- to a nontransgenic --.

Column 5,
Line 23, "development" should read -- developmental--.

Column 8,
Line 65, "such as" should read -- such an--.

Column 51,
Line 23, Claim 7, "Met_{178}" should read --Met_{176}--.

Column 52,
Line 9, Claim 12, "Leu_{189}" should read –Leu_{169}--.

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PATENT NO. 6,870,075
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FORM PTO-1050 (REV. 3-75) UNITED STATES PATENT AND TRADEMARK OFFICE

JUN 16 2005

Claim 15 (currently amended) A method for producing a non-transgenic, herbicide resistant or tolerant plant comprising:

(a)[a.] introducing into [a plant cell] plant cells a recombinagenic oligonucleobase with a targeted mutation in the EPSPS gene to produce plant cells with a mutant EPSPS gene that expresses [an] a mutant EPSPS protein that is mutated at one or more amino acid positions, said positions selected from the group consisting of Leu_{173}[;], Ala_{179}, Met_{180}, Arg_{181}, Ser_{98}, Ser_{255} and Leu_{198} in the *Arabidopsis* EPSPS protein or at an analogous amino acid residue in an EPSPS paralog;

(b)[b.] identifying a plant cell having a [mutated EPSPS gene, which encoded] mutant EPSPS protein that has substantially the same catalytic activity as compared to a corresponding wild type EPSPS protein in the presence of glyphosate; and

(c)[c.] regenerating a non-transgenic herbicide resistant or tolerant plant having a mutated EPSPS gene from said plant cell.

Claim 20 (currently amended) The method according to claim 14 in which the amino acid positions [in the *Zea mays* paralog] are selected from the group consisting of Leu_{97}, Ala_{103}, Met_{104}, Arg_{105}, Ser_{23}, Ser_{179}[,] and Leu_{122} in the *Zea mays* paralog.

Claim 21 (currently amended) The method according to claim 14 in which the amino acid positions [in the *Brassica napus* paralog] are selected from the group consisting of Leu_{169}, Ala_{175}, Met_{176}, Arg_{177}, Ser_{94}, Ser_{251} and Leu_{194} in a *Brassica sp* paralog.

Claim 22 (currently amended) The method according to claim 14 in which the amino acid positions [in the *Petunia hybrida*] are selected from the group consisting of Leu_{169}, Ala_{175}, Met_{176}, Arg_{177}, Ser_{94}, Ser_{251} and Leu_{194} in the *Petunia hybrida* paralog.

At claim 23, line 1, "plant is" has been amended to -- plant cells are -- to be in agreement with claims 14 and 15.

Claim 25 (currently amended) The method according to claim 15 in which the amino acid positions [in the *Zea mays* paralog] are selected from the group consisting of Leu_{97}, Ala_{103}, Met_{104}, Arg_{105}, Ser_{23}, Ser_{179}[,] and Leu_{122} in the *Zea mays* paralog.

Claim 26 (currently amended) The method according to claim 15 in which the amino acid positions [in the *Brassica napus* paralog] are selected from the group consisting of Leu_{169}, Ala_{175}, Met_{176}, Arg_{177}, Ser_{94}, Ser_{251} and Leu_{194} in a *Brassica sp* paralog.

Claim 27 (currently amended) The method according to claim 15 in which the amino acid positions [in the *Petunia hybrida*] are selected from the group consisting of Leu_{169}, Ala_{175}, Met_{176}, Arg_{177}, Ser_{94}, Ser_{251} and Leu_{194} in the *Petunia hybrida* paralog.

Claim 28 (currently amended) A method for producing a non-transgenic, herbicide resistant or tolerant plant comprising:

(a)[a.] introducing into [a plant cell] plant cells a recombinagenic oligonucleobase with a targeted mutation in the EPSPS gene to produce plant cells with a mutant EPSPS gene that expresses an EPSPS protein that is mutated in two amino acid positions, said positions selected from the group consisting of Thr_{178} and Pro_{182}, in the *Arabidopsis* EPSPS protein or at an analogous amino acid residue in an EPSPS paralog wherein the Thr_{178} is changed to Val or Leu and Pro_{182} is changed to Ser;

(b)[b.] identifying a plant cell having[, a mutated EPSPS gene, which cell has] substantially normal growth as compared to a corresponding wild-type plant cell in the presence of glyphosate; and

Tolerance of plants to glyphosate can be increased by introducing a mutant EPSPS gene having an alteration in the EPSPS amino acid coding sequence into the genome of the plant. Examples of some of the mutations in the EPSPS gene for inducing glyphosate tolerance are described in the following patents: U.S. Patent No. 5,310,667; U.S. Patent No. 5,866,775; U.S. Patent No. 5,312,910; U.S. Patent No. 5,145,783. These proposed mutations typically have a higher K_i for glyphosate than the wild-type EPSPS enzyme which confers the glyphosate-tolerant phenotype, but these variants are also characterized by a high K_m for PEP which makes the enzyme kinetically less efficient (Kishore et al., 1988, Ann. Rev. Biochem. 57:627-663; Schulz et al., 1984, Arch. Microbiol. 137: 121-123; Sost et al., 1984, FEBS Lett. 173: 238-241; Kishore et al., 1986, Fed. Proc. 45: 1506; Sost and Amrhein, 1990, Arch. Biochem. Biophys. 282: 433-436). Many mutations of the EPSPS gene are chosen so as to produce an EPSPS enzyme that is resistant to herbicides, but unfortunately, the EPSPS enzyme produced by the mutated EPSPS gene has a significantly lower enzymatic activity than the wild-type EPSPS. For example, the apparent K_m for PEP and the apparent K_i for glyphosate for the wild-type EPSPS from *E. coli* are 10 μM and 0.5 μM, while for a glyphosate-tolerant isolate having a single amino acid substitution of alanine for glycine at position 96, these values are 220 μM and 4.0 mM, respectively. A number of glyphosate-tolerant EPSPS genes have been constructed by mutagenesis. Again, the glyphosate-tolerant EPSPS had lower catalytic efficiency (V_{max} /K_m), as shown by an increase in the K_m for PEP, and a slight reduction of the V_{max} of the wild-type plant enzyme (Kishore et al., 1988, Ann. Rev. Biochem. 57:627-663).

Since the kinetic constants of the variant enzymes are impaired with respect to PEP, it has been proposed that high levels of overproduction of the variant enzyme, 40-80 fold, would be required to maintain normal catalytic activity in plants in the presence of glyphosate (Kishore et al., 1988, Ann. Rev. Biochem. 57:627-663). It has been shown that glyphosate-tolerant plants can be produced by inserting into the genome of the plant the capacity to produce a higher level of EPSP synthase in the chloroplast of the cell (Shah et al., 1986, Science 233, 478-481), which enzyme is preferably glyphosate-tolerant (Kishore et al.,1988, Ann. Rev. Biochem. 57:627-663).

The introduction of the exogenous mutant EPSPS genes into plant is well documented. For example, according to U.S. Patent No. 4,545,060, to increase a plant's resistance to glyphosate, a gene coding for an EPSPS variant having at least one mutation that renders the enzyme more resistant to its competitive inhibitor, *i.e.*, glyphosate, is introduced into the plant genome. However, many complications and problems are associated with these examples. Many such mutations result in low expression of the mutated EPSPS gene product or result in an EPSPS gene product with significantly lower

of recombinagenic oligonucleobases include: U.S. Patent Nos. 5,756,325; 5,871,984; 5,760,012; 5,888,983; 5,795,972; 5, 780,296; 5,945,339; 6,004,804; and 6,010,907 and in International Patent No. PCT/US00/23457; and in International Patent Publication Nos. WO 98/49350; WO 99/07865; WO 99/58723; WO 99/58702; and WO 99/40789. Recombinagenic oligonucleobases include mixed duplex oligonucleotides, non-nucleotide containing molecules taught in Kmiec II and other molecules taught in the above-noted patents and patent publications.

Citation or identification of any reference in Section 2, or any section of this application shall not be construed as an admission that such reference is available as prior art to the present invention.

3. SUMMARY OF THE INVENTION

The present invention is directed to a non-transgenic plant or plant cell having one or more mutations in the EPSPS gene, which plant has increased resistance or tolerance to a member of the phosphonomethylglycine family and which plant exhibits substantially normal growth or development of the plant, its organs, tissues or cells, as compared to the corresponding wild-type plant or cell. The present invention is also directed to a non-transgenic plant having a mutation in the EPSPS gene, which plant is resistant to or has an increased tolerance to a member of the phosphonomethylglycine family, *e.g.*, glyphosate, wherein the mutated EPSPS protein has substantially the same catalytic activity as compared to the wild-type EPSPS protein.

The present invention is also directed to a method for producing a non-transgenic plant having a mutated EPSPS gene that substantially maintains the catalytic activity of the wild-type protein irrespective of the presence or absence of a herbicide of the phosphonomethylglycine family. The method comprises introducing into a plant cell a recombinagenic oligonucleobase with a targeted mutation in the EPSPS gene and identifying a cell, seed, or plant having a mutated EPSPS gene.

Illustrative examples of a recombinagenic oligonucleobase is found in following patent publications, which are incorporated in their entirety be reference herein: U.S. Patent Nos. 5,565,350; 5,756,325; 5,871,984; 5,760,012; 5,731,181; 5,888,983; 5,795,972; 5, 780,296; 5,945,339; 6,004,804; and 6,010,907 and in International Patent No. PCT/US00/23457; and in International Patent Publication Nos. WO 98/49350; WO 99/07865; WO 99/58723; WO 99/58702; and WO 99/40789.

The plant can be of any species of dicotyledonous, monocotyledonous or gymnospermous plant, including any woody plant species that grows as a tree or shrub, any herbaceous species, or any species that produces edible fruits, seeds or vegetables, or any

nucleobases of all types. An oligonucleobase compound is a compound comprising one or more oligonucleobase chains that are complementary and hybridized by Watson-Crick base pairing. Nucleobases are either deoxyribo-type or ribo-type. Ribo-type nucleobases are pentosefuranosyl containing nucleobases wherein the 2' carbon is a methylene substituted with a hydroxyl, alkyloxy or halogen. Deoxyribo-type nucleobases are nucleobases other than ribo-type nucleobases and include all nucleobases that do not contain a pentosefuranosyl moiety.

An oligonucleobase strand generically includes both oligonucleobase chains and segments or regions of oligonucleobase chains. An oligonucleobase strand has a 3' end and a 5' end. When a oligonucleobase strand is coextensive with a chain, the 3' and 5' ends of the strand are also 3' and 5' termini of the chain.

According to the present invention, substantially normal growth of a plant, plant organ, plant tissue or plant cell is defined as a growth rate or rate of cell division of the plant, plant organ, plant tissue, or plant cell that is at least 35%, at least 50%, at least 60%, or at least 75% of the growth rate or rate of cell division in a corresponding plant, plant organ, plant tissue or plant cell expressing the wild type EPSPS protein.

According to the present invention, substantially normal development of a plant, plant organ, plant tissue or plant cell is defined as the occurrence of one or more developmental events in the plant, plant organ, plant tissue or plant cell that are substantially the same as those occurring in a corresponding plant, plant organ, plant tissue or plant cell expressing the wild type EPSPS protein.

According to the present invention plant organs include, but are not limited to, leaves, stems, roots, vegetative buds, floral buds, meristems, embryos, cotyledons, endosperm, sepals, petals, pistils, carpels, stamens, anthers, microspores, pollen, pollen tubes, ovules, ovaries and fruits, or sections, slices or discs taken therefrom. Plant tissues include, but are not limited to, callus tissues, ground tissues, vascular tissues, storage tissues, meristematic tissues, leaf tissues, shoot tissues, root tissues, gall tissues, plant tumor tissues, and reproductive tissues. Plant cells include, but are not limited to, isolated cells with cell walls, variously sized aggregates thereof, and protoplasts.

Plants are substantially "tolerant" to glyphosate when they are subjected to it and provide a dose/response curve which is shifted to the right when compared with that provided by similarly subjected non-tolerant like plant. Such dose/response curves have "dose" plotted on the X-axis and "percentage kill", "herbicidal effect", etc., plotted on the y-axis. Tolerant plants will require more herbicide than non-tolerant like plants in order to produce a given herbicidal effect. Plants which are substantially "resistant" to the glyphosate exhibit few, if any, necrotic, lytic, chlorotic or other lesions, when subjected to

invention can be practiced with mixed duplex oligonucleotides having two or more types of RNA-type nucleotides. The function of an RNA segment may not be affected by an interruption caused by the introduction of a deoxynucleotide between two RNA-type trinucleotides, accordingly, the term RNA segment encompasses such an "interrupted RNA segment." An uninterrupted RNA segment is termed a contiguous RNA segment. In an alternative embodiment an RNA segment can contain alternating RNase-resistant and unsubstituted 2'-OH nucleotides. The mixed duplex oligonucleotides preferably have fewer than 100 nucleotides and more preferably fewer than 85 nucleotides, but more than 50 nucleotides. The first and second strands are Watson-Crick base paired. In one embodiment the strands of the mixed duplex oligonucleotide are covalently bonded by a linker, such as a single stranded hexa, penta or tetranucleotide so that the first and second strands are segments of a single oligonucleotide chain having a single 3' and a single 5' end. The 3' and 5' ends can be protected by the addition of a "hairpin cap" whereby the 3' and 5' terminal nucleotides are Watson-Crick paired to adjacent nucleotides. A second hairpin cap can, additionally, be placed at the junction between the first and second strands distant from the 3' and 5' ends, so that the Watson-Crick pairing between the first and second strands is stabilized.

The first and second strands contain two regions that are homologous with two fragments of the target EPSPS gene, *i.e.*, have the same sequence as the target gene. A homologous region contains the nucleotides of an RNA segment and may contain one or more DNA-type nucleotides of connecting DNA segment and may also contain DNA-type nucleotides that are not within the intervening DNA segment. The two regions of homology are separated by, and each is adjacent to, a region having a sequence that differs from the sequence of the target gene, termed a "heterologous region." The heterologous region can contain one, two or three mismatched nucleotides. The mismatched nucleotides can be contiguous or alternatively can be separated by one or two nucleotides that are homologous with the target gene. Alternatively, the heterologous region can also contain an insertion or one, two, three or of five or fewer nucleotides. Alternatively, the sequence of the mixed duplex oligonucleotide may differ from the sequence of the target gene only by the deletion of one, two , three, or five or fewer nucleotides from the mixed duplex oligonucleotide. The length and position of the heterologous region is, in this case, deemed to be the length of the deletion, even though no nucleotides of the mixed duplex oligonucleotide are within the heterologous region. The distance between the fragments of the target gene that are complementary to the two homologous regions is identically the length of the heterologous region when a substitution or substitutions is intended. When the heterologous region contains an insertion, the homologous regions are thereby separated in